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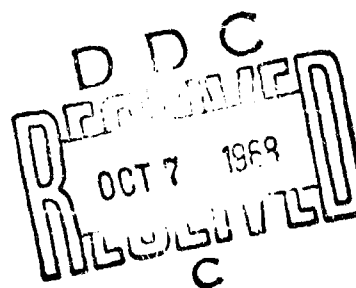
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FURTHER RESEARCH ON DETERMINATION OF DNA IN BLOOD
AS AN INDEX OF RHEUMATIC PROCESS ACTIVITY

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In diseases with a systemic affliction of the connective tissues, such as rheumatic fever and progressive polyarthritis, the intimate mechanisms of metabolic disorders in general, and of protein metabolism disorders in particular, have not yet been clarified adequately, and they require further study. The relationship of these changes to the innermost disorders of cell metabolism has not been established accurately, although the significant role that nucleoproteids and nucleic acids play in the synthesis and metabolism of proteins is known. It has been observed that various derivatives of the nucleic acids are components of the ferments and co-ferments. The close relationship between the nucleic acids and antigen structures also has been pointed out.

The study of nucleic acid metabolism in various pathological processes is a new and timely problem. The disorders and disorganization of the system of connective tissues may depend to a considerable extent on the changes in nucleic acid metabolism.

In patients suffering from rheumatic fever and progressive polyarthritis the nucleic acid metabolism is relatively unexplored. There are only individual reports on the quantitative content of nucleic acids in the connective tissues, as a function of rheumatic process activity (R. Ptasheskas, M. Ye. Kurmayeva). In our previous investigations (1963), and also in the works of V. P. Kaznacheyev et al (1964), and of I. Danis and B. Yusyavichyute (1964), an attempt was made to study quantitatively the DNA content in the blood of patients suffering from rheumatic fever.

In this work further research was done on the DNA content in the blood (in the formed components of the nuclei) of rheumatic fever patients

in various phases of the rheumatic process, dynamically, before and after antirheumatic therapy; and also in the blood of patients suffering from progressive polyarthrititis and chronic tonsillitis. In addition, we studied the DNA content of the tonsil tissue that the Otorhinolaryngology Clinic of the Volgograd Medical Institute (clinic headed by Professor Z. Volfson) supplied us after tonsillectomies on patients suffering from chronic tonsillitis.

An attempt was made also to compare these data with certain indicators that characterize the connective tissue system and give an indication of the acuteness and activity of the process (diphenylamine reaction, determination of sialic acid). A comparative study was made of the quantitative DNA content and of the data from the determination of the anti-streptolysin O titers, C-reactive protein, and fibrinogen.

In our investigations we used Persini's modified method for the determination of DNA, spectrophotometrically, on an SF-2m spectrophotometer with automatic recording. The calibration curve was constructed on the basis of the DNA standard supplied by the Immunological Laboratory, Institute of Rheumatism, Academy of Medical Sciences USSR.

In each determination the DNA content was computed in micrograms per 10,000 leukocytes and reduced to 1.0 milliliter of blood. When the DNA content of tonsil tissue was studied, it was computed in micrograms per one gram of tissue.

In all we investigated 311 patients. These included 160 with rheumatic fever, 29 with progressive polyarthrititis, and 122 with chronic tonsillitis. Of the 122 chronic tonsillitis patients, the DNA content of the tonsil tissue was studied in 57; the DNA content of the blood, in 44; and the DNA content of both the blood and tonsil tissue, in 21. There were 185 females and 126 males. The breakdown by age was the following: from 13 to 25, 120 patients; from 26 to 35, 115 patients; from 36 to 45, 41 patients; from 46 to 55, 29 patients; and 56 or older, six patients.

An overwhelming majority of the patients, then, were youths or persons of middle age. In addition, we investigated a control group of 18 healthy persons, from 20 to 31 years of age.

There were 125 patients in the active phase of rheumatic fever, and 35 in the inactive phase. Of the 125 rheumatic fever patients in the active phase, nine had predominantly articular symptoms, and 116 had rheumatic carditis. The course was acute and subacute (activities III and II) in 19 patients; chronic (activity I) in 67; constantly relapsing in 35; and latent (activity I) in four. The attack of rheumatic fever was the first for 11 patients, and recurrent for 114 who had developed rheumatic heart disorders. Among the 114 patients with some valvular disorder, 24 had first-degree circulation insufficiency, 68 had second-degree insufficiency, and two had third-degree circulation insufficiency. We observed 20 patients during complete compensation.

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Among the 29 patients with progressive polyarthritis, the disease was acute in five patients and subacute in 24. In eight patients the symptoms were mostly exudative, based on joint deformation. There were 21 patients in the exudative proliferating phase of the process, with predominantly proliferative symptoms. Focal infection was found in 13 patients.

First we attempted to determine the DNA content in the 18 healthy persons comprising the control group. It was found that the DNA content varied from 0.03 to 0.15 μg per 10,000 leukocytes. In 16 of the 18 healthy persons, the DNA content did not exceed 10 [sic] μg per 10,000 leukocytes. Reduced to 1.0 ml of blood, the DNA content in persons of the control group ranged from 19 to 99 $\mu\text{g}/\text{ml}$.

Different were the results obtained in the case of rheumatic fever patients. Patients in the active phase with an acute or subacute course (activities III and II) of the disease usually showed (16 of the 19 patients) a high DNA content, much higher than the healthy persons comprising the control group (from 0.16 to 0.90 μg per 10,000 leukocytes; or reduced to one millimeter of blood, from 271 to 885 $\mu\text{g}/\text{ml}$). The difference between the mean values of the DNA content and the patients with an acute or subacute course (activities III and II) and healthy persons was clearly significant (Table 1). After antirheumatic treatment, before release from the ward, in most of the investigated patients (ten patients were examined dynamically) the DNA content actually did not differ from normal (0.07 to 0.11 μg per 10,000 leukocytes; reduced to one milliliter of blood, 43 to 91 $\mu\text{g}/\text{ml}$, and in one patient 165 $\mu\text{g}/\text{ml}$). In other words, no actual difference was found in the DNA content between patients prior to their release from the ward, and healthy persons (Table 1).

An increased DNA content was found in patients with a chronic course (activity I) of the disease (0.18 to 0.54 μg per 10,000 leukocytes in 60 of the 67 patients, or 138 to 434 $\mu\text{g}/\text{ml}$). In seven patients the DNA content was found to be normal (47 to 78 $\mu\text{g}/\text{ml}$). It must be emphasized that in most patients who initially had a high DNA content (in 49 of the 60 patients with an acute course), this indicator did not become normal before their release from the ward (the DNA content was 0.18 to 0.38 μg per 10,000 leukocytes, see Table 1). In these patients the DNA content reduced to one milliliter of blood (123 to 293 $\mu\text{g}/\text{ml}$) likewise did not become normal.

Examination of the patients with a continuously relapsing rheumatic process showed that 21 of the 35 patients had a DNA content corresponding to the minimum value obtained in healthy persons (0.03 to 0.05 μg per 10,000 leukocytes) or even lower (0.01 and 0.02 μg per 10,000 leukocytes). It is important to emphasize that in the case of leukopenia, which occurred in many patients of this group, there is a distinct DNA depletion of the blood (13 to 27 $\mu\text{g}/\text{ml}$). In five of the afore-mentioned 35 patients the recorded DNA values in the blood were normal (68 to 75 $\mu\text{g}/\text{ml}$), and in nine patients the recorded values exceeded the maximum

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Table 1

DNA Content, $\mu\text{g}/10,000$ Leukocytes
(Statistical Data)

Nosologické formy (I)		Max.	Min. *	M	Sigma \pm	m \pm	Koeficient spolehlivosti (t) při srovnání se zdravými osobami
(A)	Rheumatická horečka						(II)
(1)	Aktivní fáze, akutní či subakutní průběh (aktivita III a II);						
(a)	při nástupu	0,90	0,09	0,418	0,221	0,032	6,4
	před propuštěním (C)	0,30	0,07	0,121	0,065	0,0218	1,7
(2)	Aktivní fáze, chronický průběh (aktivita I)						
(a)	při nástupu	0,54	0,08	0,337	0,122	0,0149	16,8
	před propuštěním (C)	0,38	0,04	0,221	0,098	0,0111	10,3
(3)	Inaktivní fáze	0,28	0,05	0,11	0,067	0,011	1,9
	Progresivní polyartritida	1,73	0,10	0,404	0,345	0,0639	5,0
	Chronická tonsilitida	0,43	0,04	0,100	0,065	0,008	1,6
	Zdraví lidé	0,16	0,03	0,053	0,0305	0,074	—
	Chronická tonsilitida (v tkáni mandlí, v gama na 1 g tkáně)	1500	40	266,153	253,979	29,895	8,3
	Lidé, zahynoucí při silničních nehodách (v tkáni mandlí, v gama na 1 g tkáně)	40	8	16,333	10,965	4,917	—

Key to Table 1:

I - nosological forms; II - reliability factor (t)
in comparisons with
healthy persons;

- A. Rheumatic fever
 - 1. Active phase, acute or subacute course (activities III and II)
 - a. On admission
 - b. Before release
 - 2. Active phase, chronic course (activity I)
 - a. On admission
 - b. Before release
 - 3. Inactive phase
- B. Progressive polyarthrititis
- C. Chronic tonsillitis
- D. Healthy persons
- E. Chronic tonsillitis (in tonsil tissue, $\mu\text{g}/\text{g}$ of tissue)
- F. Victims of traffic accidents (in tonsil tissue, $\mu\text{g}/\text{g}$ of tissue)

form (0.17 to 0.96 μg per 10,000 leukocytes). Because of leukopenia that occurred in four out of these nine patients, and when controlling the results of the examinations by reduction to one milliliter of blood, this increase in the DNA content of the leukocytes cannot express an absolute increase in the DNA content of the blood (reduced to one milliliter, 87 to 95 $\mu\text{g}/\text{ml}$). Even in the course of further study, the DNA content hardly changed in patients with a continuously relapsing form of the disease.

In three of the four patients with a latent course (activity I) of the disease, an increased DNA content of the blood was recorded (0.19 to 0.93 μg per 10,000 leukocytes), but a normal content in the remaining case (0.12 μg per 10,000 leukocytes).

A comparative analysis showed that the highest DNA content occurs in rheumatic fever patients in the active phase, with predominantly articular symptoms of the disease.

We were unable to find any statistically significant relationship between the DNA content and the degree of circulation insufficiency.

Further study of the DNA content in rheumatic fever patients in the inactive phase produced the following findings. In 25 of the 35 rheumatic fever patients in the inactive phase, the DNA content per 10,000 leukocytes did not differ from the values obtained in healthy persons (0.05 to 0.15); also the absolute DNA content of the blood (35 to 91 $\mu\text{g}/\text{ml}$) did not differ from the norm. In ten patients the DNA content of the blood was increased (0.16 to 0.28 μg per 10,000 leukocytes). But we were unable to establish with sufficient reliability an increase in the DNA content in all rheumatic fever patients in the inactive phase (Table 1).

The examination of progressive polyarthritides patients established in the five acute patients and in 17 of the 24 subacute patients a tendency toward an increase in the absolute DNA content of the blood in general and in the DNA content of the leukocytes (0.18 to 1.73 μg per 10,000 leukocytes; reduced to one milliliter of blood, 172 to 1470 $\mu\text{g}/\text{ml}$). In seven of the patients with a subacute course the DNA content of the leukocytes and the DNA content reduced to one milliliter of blood were normal as compared with healthy persons (0.10 to 0.14 μg per 10,000 leukocytes; 69 to 108 $\mu\text{g}/\text{ml}$). As evident from the table, a reliable difference in the average value of the DNA content was found between progressive polyarthritides patients and healthy persons.

An analysis of the examination results of 65 chronic tonsillitis patients showed in 42 of them a normal DNA content in the leukocytes (0.04 to 0.15 μg per 10,000 leukocytes) and also in a reduction to one milliliter of blood (24 to 96 $\mu\text{g}/\text{ml}$). In 23 patients the DNA content per 10,000 leukocytes was increased (0.16 to 0.43 μg), and so was the DNA content reduced to one milliliter of blood (105 to 342 μg). But in general, we did not find a reliable difference in the average values between patients and healthy persons (Table 1).

We studied also the DNA content in the tonsil tissue of 78 chronic tonsillitis patients. The DNA content ranged from 40 to 1500 μ g per gram. For a comparison, in six instances we investigated tonsil tissue from fresh cadavers of traffic accident victims. The DNA content was low and appeared only in the minimal optical density.

The difference between the mean values appears entirely real and reliable (Table 1).

A comparative analysis showed a direct correlation between the DNA content of the blood and C-reactive protein. In 16 rheumatic patients with an acute or subacute course of the disease (activities III and II) who showed high DNA values, we found C-reactive protein. A strong positive reaction (+++, +++, and more) was observed in ten patients; and a positive reaction (++) in six patients. After antirheumatic therapy and before release from the ward, C-reactive protein in these patients disappeared, parallel with the return of the DNA content to normal.

C-reactive protein was established also in 57 of the 60 rheumatic fever patients with a chronic course (activity I), in whom the DNA content of the blood was increased. Here the reaction was strongly positive in 15 patients, positive in 13, and weakly positive (+) in 29. In three of the 60 patients with an increased DNA content, no C-reactive protein was determined in the blood. It must be emphasized that in seven rheumatic fever patients with a chronic course and a normal DNA content, the test for C-reactive protein was negative.

Among the 21 patients who had a continuously relapsing form of rheumatic fever and an absolute decrease in the DNA content of the blood, the test for C-reactive protein was weakly positive in 14 patients, and positive in four. No C-reactive protein was found in the remaining three patients. In none of these cases was there a strong positive reaction.

A direct correlation was found between the DNA content of the blood and the antistreptolysin O titers of rheumatic fever patients. Higher antistreptolysin O titers were obtained in seven tests of blood with an increased DNA content, from patients with an acute or subacute course of rheumatic fever (activities III and II). Among nine patients who had the continuously relapsing form of the disease and an increased DNA content of the blood, higher antistreptolysin O titers were found in six cases, and normal titers in the remaining three cases. Among the three patients with a normal DNA content, normal antistreptolysin O titers were recorded in two cases.

An analysis of the results of the examinations showed a parallelism between the DNA content of the blood and the fibrinogen content of the blood plasma. In 14 of the 16 patients with an acute or subacute course of the process (activities III and II) and an increased DNA content, the fibrinogen content of the blood plasma was found to be increased (from 0.55 to 0.75 g%). A multiplication of the fibrinogen in the

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plasma was established in 52 of the 60 patients who suffered from the chronic course of the disease (activity I) and had an increased DNA content in their blood. It must be emphasized that prior to the release of these patients from the ward and parallel with the failure of the DNA content to return to normal, in most of these patients (in 49 of the 60 with an initially increased DNA content) the high fibrinogen content of the plasma remained (in 43 patients, 0.52 to 0.65 g%).

In all nine of the patients with a continuously relapsing form of the disease and an absolute decrease in the DNA content of the blood, the fibrinogen content of the blood plasma was increased (0.50 to 0.70 g%).

A less pronounced correlation was found between the DNA content of the blood and the values of the diphenylamine reaction. (The micromethod of the diphenylamine reaction was used, as described by Z. I. LarSKIY in 1957, and recommended by the Institute of Rheumatism, Academy of Medical Sciences USSR, in its "Methodological Instructions," in 1961.) Only nine of the 16 rheumatic fever patients in the active phase with an acute or subacute course (activities III and II) and an increased DNA content were the values of the diphenylamine reaction increased (optical density 0.245 to 0.550). In seven patients the reaction was normal (0.130 to 0.212). An adequate correlation was not established for patients suffering from the chronic course of the disease (activity I). Increased values of the diphenylamine reaction (0.228 to 0.398) were found in only 40 of the 60 patients with an increased DNA content. Normal values of the diphenylamine reaction were recorded only in three of the seven patients in this group who had a normal DNA content in the blood. In four of these seven patients, at a normal DNA content, the diphenylamine reaction was positive (0.228 to 0.842).

No parallelism between the DNA content and the values of the diphenylamine reaction was found in patients suffering from the continuously relapsing form of the disease. Of 21 patients in this group with a low absolute DNA content in the blood, higher diphenylamine values were obtained only for seven patients (0.230 to 0.425), and in 14 the diphenylamine reaction did not depart from the norm (0.125 to 0.210). Thus the comparative analysis showed that the determination of the DNA content in the blood is more indicative than the diphenylamine value in the case of rheumatic fever patients in the active phase, with a continuously relapsing form of the disease.

In rheumatic fever patients in the active phase we were unable to find a statistically reliable correlation between the DNA content in the blood and the sialic acid. (The blood was tested for sialic acid according to the generally employed methods recommended by the Institute of Rheumatism, Academy of Medical Sciences USSR, in the "Methodological Instructions," 1961.) The quantitative value of sialic acid (in units of optical density) was increased (0.240 to 0.458) only in 62 of the 109 patients whose DNA content in the blood showed a distinct departure from the norm.

Summary:

The following conclusions may be drawn from the results of the investigation:

1. In rheumatic fever patients with an acute, subacute, chronic, and latent course of the disease (activities III, II and I) the DNA content of the leukocytes is increased in most cases, even when reduced to one milliliter of blood. Parallel with the increase of the DNA content, however, in patients suffering from the continuously relapsing form of the disease there is more often an absolute drop in the amount of DNA in the blood, and this drop hardly changes in the course of subsequent examinations.

2. In most patients suffering from an acute or subacute form of the disease (activities III and II) the DNA content returns to normal as the activity of the process diminishes. In patients suffering from a chronic course of the disease (activity I), in most cases this index does not return to normal before the patients' release from the ward.

3. A higher DNA content in the blood is found in most patients suffering from progressive polyarthritides, especially in the acute course of the process.

4. In more than 50 percent of the investigated chronic tonsillitis patients the DNA content of the leukocytes is normal, and also when reduced to one milliliter of blood. In the tonsil tissue the DNA content was increased.

5. The investigation established a direct correlation between the DNA content of the blood and the C-reactive protein, antistreptolysin O titers, and fibrinogen. The correlation with the values of the diphenylamine reaction and sialic acid test was less pronounced.

In patients in the active phase of the rheumatic process an increased DNA content was found more frequently than an increase in the values of the diphenylamine reaction and sialic acid.